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Malignant Histiocytosis

A Phenotypic and Genotypic Investigation

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Ten cases of malignant histiocytosis (MH) were evaluated for clinical and histopathologic features, phenotype, and rearrangement of T cell receptor (TCR) β, γ, and α and immunoglobulin (Ig) genes (7/10). All cases were HLA-DR+ and CD30-positive. Four cases had molecular evidence of T cell lineage such as TCR β, γ, and α rearrangements, and one additional case synthesized the cytoplasmic TCR β chain. The remaining five cases did not show unequivocal T, B, natural killer (NK) cell, or macrophagic origin, and three of them had germline TCR and Ig genes. Ultrastructural analysis was not helpful for the definition of the cell lineage. Most myelomonocytic markers (MAC387, CD13, CD14, CD64, CD68) were either negative on the MH cells or were expressed on cells with rearranged TCR gene. Precursor (CD34, CD7) and NK (CD16, CD56, and CD57) cell markers were not found. The lineage of a number of cases of MH remains unresolved. (Am J Pathol 1990, 136:1009–1019)

Malignant histiocytosis (MH), according to the World Health Organization (WHO) guidelines,[1] is a systemic progressive proliferation of neoplastic cells resembling histiocytes and frequently displaying phagocyting activity. The disease was renamed[2] from the former term of "histiocytic medullary reticulosis."[3] The disease may occasionally appear as a discrete involvement of lymph nodes or other organs such as the gastrointestinal tract, where it displays peculiar clinicopathologic features.[4] Recently, some doubt has been cast on the actual histiocytic nature of the proliferating malignant cell, by virtue of technologies appropriate for the analysis of the genes involved in lymphoid cell differentiation and because of the availability of new immunologic tools. The demonstration of T cell gene rearrangement in MH of the intestine[4] and the identification by the use of the Ki-1 antibody[5] of a new type of large cell, mostly T-cell–derived, anaplastic non-Hodgkin lymphoma (NHL) resembling MH, prompted this evaluation of 10 cases of *bona fide* MH by means of a combined phenotypic and genotypic approach.

Materials and Methods

On the basis of available tissue, 10 cases were selected with nodal (seven cases) and extranodal (three cases) malignant cell proliferation in which the diagnosis of malignant histiocytosis was done according to the usual histologic diagnostic criteria.[1,3]

The biopsy material of each patient was fixed in 15% formalin, embedded in paraffin, and the sections were stained with hematoxylin and eosin (H & E), Giemsa, Pas-hematoxylin, and Gomori's silver stain for reticulin fibers.

In all cases, the biopsy contained more than 10% of large neoplastic CD30+, EMA+, CD25+ cells. In five cases there was a significant proportion of non-neoplastic cells admixed with the atypical cell population.

Immunohistochemistry

Fresh biopsies were frozen in liquid nitrogen-precooled isopentane (BDH, Poole, United Kingdom) and stored at −80°C.

Cryostat sections (4 μ) were collected on clean glass slides, dried overnight, fixed in acetone (Merck, Darmstadt, West Germany) for 10 minutes and hydrated in TRIS-buffered saline (TBS). Conventional indirect immunohistochemistry was performed according to Stein et al.[6] Briefly, slides were incubated overnight with the first anti-

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Table 1. *List of Antibodies Used*

WS	MAb	Source	MW (kDa)	Typical reactivity
CD1a	NA1/34	*	49–12	Thymus, Langerhans and IDR cells.
CD1b	NUT-2	*	45–12	Thymus and IDR cells
CD1c	L161	*	43–12	Thymus, B cells and subpop. of Langerhans cells
CD2	MT110	*	50	LFA-3 receptor, pan T cells
CD3	Leu4	†	19–29	TCR associated multimeric complex, pan T cells
CD4	Leu3	†	55	MHC class II-restricted receptor on Th and monocytes
CD5	UCHT3	*	67	T, B-CLL cells and platelets
CD6	T12	*	120	Mature T cells
CD7	WT1	‡	40	T cells and, rarely, myeloid leukemias
CD8	OKT8	§	32–33	MHC class I-restricted receptor on Ts
CD10	J5	‖	100	B precursor cells
CD11a	Ts1/22.1	*	170	LFA αL heavy chain
CD11b	OKM1	§	165	LFA αM heavy chain
CD11c	3.9	*	150	LFA αX heavy chain
CD13	MCS2	*	150	Myeloid cells
CD14	UCHM1	¶	55	Mature monocytes
CD16	CLB-Fcgranl	*	50–60	FcγR-III, NK, PMNs
CD18	Ts1/18.1	*	95	LFA associated β chain
CD19	B4	‖	95	B and monocytic precursor cells
CD20	B1	‖	35	Mature B cells
CD22	To15	#	135	B cells
CD23	MHM6	*	45	LMW BCGF-R, B cells
CD25	Tac1	*	55	IL2 receptor, activated T, B, Histiocytes
CD28	Kolt-2	*	44	T cells
CD30	Ki-1	#	90	Activation antigen on T and B cells
	BerH2	#		Activation antigen on T and B cells
CD34	MY10	†	115	Precursor cells
CD36	OKM5	§	88	Monocytes, endothelium
CD45	F10894	*	180–220	Leukocyte common antigen
	9.4	*	180–220	Leukocyte common antigen
	2B11	*	180–220	Leukocyte common antigen
CD45Ra	F8.11.13	*	220	B restricted LCA, B cells, Ts, NK cells
	10G3	*	220	B restricted LCA, epitope 4
CD45Rb	PD7/26	*	190–220	Leucocyte common antigen
CD45RO	UCHL1	¶	180	T restricted LCA, T cells, monocytes
CD56	N901	¶	135	N-CAM, NK
CD57	Leu7	†	110	NK
CD64	10.1	*	71	FcγR-I, activated macrophage
CD68	Ki-M6	*	60	pan macrophage
	EBM11	*		pan macrophage
–	AA3.84	**	29–33	HLA-DR
–	Ki-M8	*		Activated macrophage
–	βF1	††	40	TCR β chain
–	TCRδ1	††	41–44	TCR δ chain
–	δTCS1	††	41	TCR δ chain
–	lysozyme	#		Lysozyme (polyclonal)
–	MAC387	#	12–14	Subpopulation of macrophages
–	S-100	#	20	S-100 $\alpha\beta$ (polyclonal)

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Workshop Cluster Differentiation number; MW(kd), molecular mass (kd); IDR, interdigitating reticulum cells; Th, T helper cells; Ts, T suppressor cells; LMW BCGF-R, low-molecular weight B-cell growth factor receptor; NK, natural killer cells.

body, washed, and stained sequentially with rabbit anti-mouse immunoglobulin (Dako, Glostrup, Denmark; 1:50 in TBS) and alkaline phosphatase antialkaline phosphatase (APAAP) complexes (Dako; 1:50 in TBS). The procedure was repeated twice. Then the slides were developed with Naphtol AS-BI Phosphate (Sigma, St. Louis, MO) and neofuchsin,[6] counterstained with hematoxylin, and mounted.

Double labeling of cells was performed by first staining for Ki-67 with biotin-conjugated horse anti-mouse antibody (Vector, Burlingame, CA; 1:200 in PBS) followed by avidin-biotin-peroxidase complexes (ABC; Vector) and then developed with aminoethyl carbazole (AEC; Sigma). The slides then were incubated with the second primary antibody and immunostained with two rounds of APAAP.

Table 2. *Clinical Data*

No.	Sex	Age (yrs)	Fever	Weight loss	Sweats	Enlarg. LN	Liver	Spleen	Skin	Stage	Survival (mos)
1	F	12	+	+	+	+	+	+	−	III° B	34 AW
2	F	18	+	+	+	+	+	−	+	IV° B	36 AW
3	F	46	+	+	+	+	+	+	+	IV° B	20 DoD
4	M	20	+	−	−	+	−	−	+	IV° B	39 DoD
5	F	11	+	+	−	+	−	−	+	IV° B	21 DoD
6	M	15	+	−	−	+	−	−	−	III° B	19 AW
7	M	47	−	−	−	+	+	−	−	III° A	65 AW
8	F	13	+	−	−	−	−	−	+	I° B	30 AW
9	M	4	−	+	+	+	−	−	−	II° B	10 AW
10	M	9	−	+	+	+	+	+	−	III° B	19 AW

LN, lymph nodes; AW, alive and well; DoD, dead of disease.

The latter reaction was developed with Fast Blue BB diazonium salt (Sigma).

Paraffin sections were dewaxed with two changes of xylene and brought to phosphate-buffered saline (PBS) with a graded alcohol series. They then were incubated with the primary antibodies as above, counterlabeled with a biotin-conjugated secondary antibody, and developed with a modified ABC system.[7] Double labeling for CD30 and MAC387 was performed by first staining for CD30 (AEC), then trypsin-digesting the slides (15′ at 37°C in a solution of 0.1% trypsin in PBS), and finally staining for MAC387 or lysozyme (Fast Blue). This method was found to be the treatment of choice for optimal immunostaining of both CD30 (Trypsin-labile) and MAC387 or lysozyme (Trypsin-enhanced).

The list and the specificity of the antibodies used is given in Table 1.

A positive score was assigned to each marker when it was demonstrated on at least 10% of malignant cells.

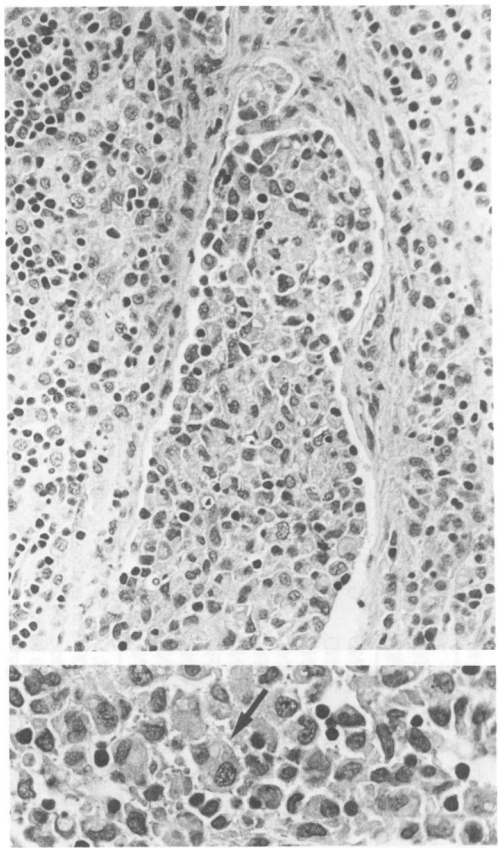

Figure 1. *Case 1. Lymph node. Enlarged medullary sinus filled with atypical histiocytes (×250).* **Inset:** *At higher magnification, there is evidence of erythrophagocytosis (arrow) (H&E, ×500).*

Molecular Analysis of Gene Rearrangement

High-molecular-weight DNA was prepared, blotted, and hybridized as previously described.[8] Filters were washed

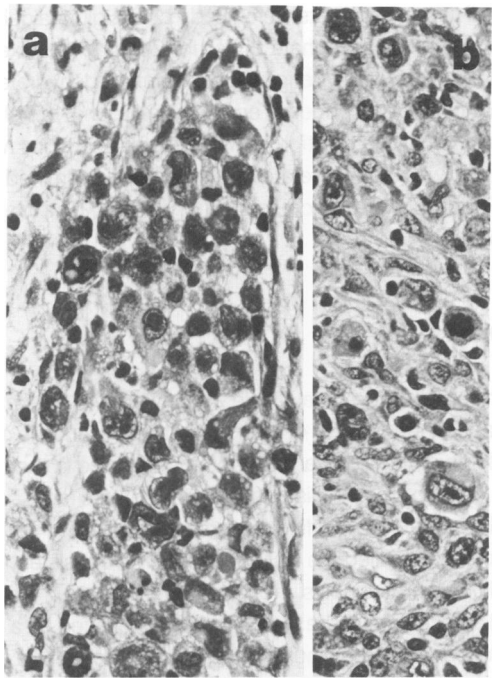

Figure 2. *Case 2. Lymph node.* **A:** *Medullary sinus containing atypical cells with irregularly shaped nuclei (×500);* **B:** *Variably sized abnormal cells with distinct nuclear membrane and large nucleoli (H&E ×400).*

three times at room temperature in 2 × sodium chloride, 0.1% sodium dodecyl sulphate (SDS) for 5 minutes, and twice at 42°C in 0.1 × sodium citrate, 0.1% SDS for 20 minutes, and then autoradiographed for various periods of time at −70°C with intensifying screen.

The probe used to study immunoglobulin heavy chain gene was a Bam HI/Hind III fragment of a clone (J_H) specific for the J region.[9] For the analysis of kappa and lambda immunoglobulin (Ig) genes, two probes specific for the C_k and C_λ regions[9,10] were used. All these probes were provided by Dr. P. Leder (Cambridge, MA).

The probe used to detect T cell receptor β chain gene rearrangement was Jurkat 2,[11] provided by Dr. T. W. Mak (Toronto, Canada), who also donated a γ gene-specific cDNA that includes V, J, and C segments (T. W. Mak, written personal communication, July 1, 1986). We used also the M13H60 γ gene probe provided by Dr. Rabbitts.[12] T cell receptor (TCR) α gene was evaluated with two DNA probes: K40D[13] (donated by Dr. D. Mathieu-Mahul, Paris, France) and PY14[14] (Dr. T. W. Mak, Toronto, Canada).

The detection limit of nongermline bands according to our procedure is 5% of clonal cells; all cases contained a minimum of neoplastic cells beyond this value.

Electron Microscopy

Small tumor fragments were fixed in 2.5% glutaraldehyde in Sorensen's phosphate buffer for 2 hours, postfixed in 1% osmium tetroxyde in Millonig's buffer, dehydrated in ethanol, and embedded in epoxy resin (Epon-812 Polaron Equipment Ltd., Watford, UK). Ultrathin sections were stained with uranyl acetate lead citrate and examined with a Philips EM 300 microscope (Eindhoven, The Netherlands).

Results

Clinicopathologic data (Table 2) showed a 1:1 sex ratio, an age range of 11 to 47 years, and the presence in most patients of the signs and symptoms reported in the classic descriptions.[2,3] Histopathologic features and genotypic and phenotypic findings are summarized in Table 3. All cases revealed the histologic patterns consistent with malignant histiocytosis. In the seven nodal cases, the normal architecture of the lymph node was effaced by a proliferation within the sinuses of atypical noncohesive cells with irregular hyperchromatic nuclei with dense chromatin and thick nuclear membrane (Figures 1, 2); the nucleoli were prominent and basophilic or amphophilic (Figure 3). In some cases, cohesive masses of atypical cells showed

Table 3. *Genotypic and Phenotypic Analysis of Malignant Histiocytosis*

N	Site	TCR beta	TCR gamma	TCR alpha	JH	CD30	CD25	EMA	βF1	TCRδ1 δTC51	CD4	CD5	CD2	UCHL1	MAC 387	CD13	CD14	CD64	EBM11	Lys.	LFA Beta	LFA aL	LFA aX	LFA aM
1	N	G	G	ND	G	+	+	+	−	−	+	−	−	+	−	+	−	−	−	−	+	−	−	−
2	N	ND	ND	ND	ND	+	+	+	−	−	+	+	+	+	−	−	−	−	−	−	+w	+	+	−
3	N	G	G	ND	G	+	+	+	−	−	−	−	−	+w	−	+	−	−	−	−	−	−	−	−
4	N	G	G	ND	G	+	+	+	−	−	−	+	ND	−	−	−	−	−	−	+	+w	−	−	−
5	Ex	R	R	R	G	+	+	+	−	−	+	−	−	+w	+	+	−	−	−	−	+w	−	+w	−
6	Ex	R	R	ND	G	+	+	+	−	ND	−	−	−	+	−	−	−	−	−	+	−	−	−	−
7	N	R	R	R	G	+	+	+	−	−	+	−	−	+	−	−	−	−	−	−	−	−	−	−
8	Ex	R	ND	ND	G	+	−	+	+	ND	+	+	−	−	−	+w	+	−	−	−	+	+	+	−
9	N	ND	ND	ND	ND	+	+	+	−	ND	+	−	−	+w	−	+	−	−	−	−	ND	ND	ND	ND
10	N	ND	ND	ND	ND	+	+	−	+	ND	+	+	ND	+	−	+	−	−	+	−	ND	ND	ND	ND

Legend: N, nodal; Ex, extranodal; Lys., lysozyme-positive cells; ND, not done; G, germline; R, rearranged; w, weak.
All cases were positive for HLA-DR and negative for the following CDs: 1a, 1b, 1c, 3, 6, 7, 8, 10, 19, 20, 22, 23, 45Ra, and the nonclustered MAb KiM8.
Cases 1 to 8 were also negative for CDs: 28, 36, w65, and 41a.
Cases 1 to 5 and 7 were negative for CDs: 16, 34, 56, 57.
Cases 1 to 6, 9 and 10 were negative for S-100, neutrophil elastase NP57, CD20-like L26 antibodies (on paraffin sections).

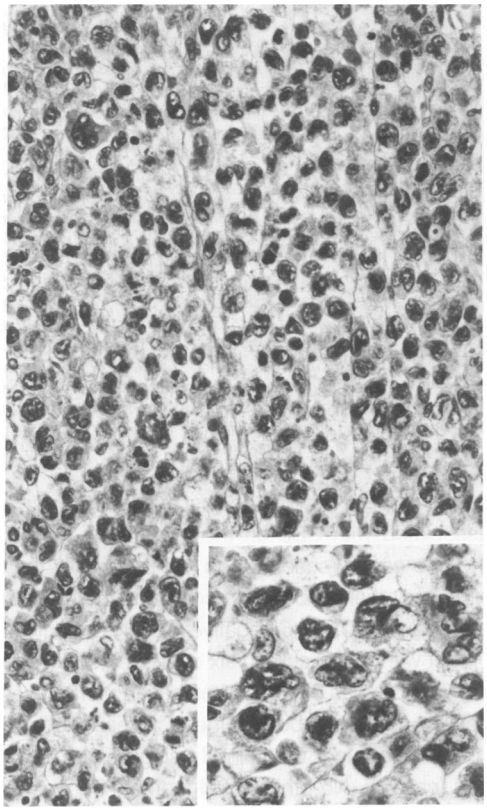

Figure 3. *Case 7. Lymph node. Proliferation of atypical cells within dilated medullary sinuses (×250).* **Inset:** *Bizarre and lobated nuclei with dense chromatin (H&E×500).*

a destructive growth pattern (Figure 4). The mitotic index was always high. Phagocytosis, even though quantitatively variable, was present in seven cases (1 to 3, 5, 7 to 9). In three cases, the neoplastic proliferation consisted of infiltrates or aggregates of neoplastic cells located in the dermis and/or subcutaneous fatty tissue (Figure 5).

Gene Rearrangement Studies

Case 5 to 7 had one (case 7) or two (cases 5 and 6) rearranged TCR β genes (Figure 6A). All cases had TCR γ gene rearrangement as well, as demonstrated by at least one of the two probes used (Figure 6B). In cases 5 and 7, TCR α gene rearrangement also could be demonstrated (Figure 7). Case 8 was shown to be rearranged at the Cβ2 TCR locus in a separate investigation (MA Pierotti, personal communication).

Cases 1, 3, and 4 had germline TCR β and TCR γ genes. Cases 1, 3, and 5 to 8 had germline JH genes.

Cases 2, 9, and 10 could not be investigated for gene rearrangement.

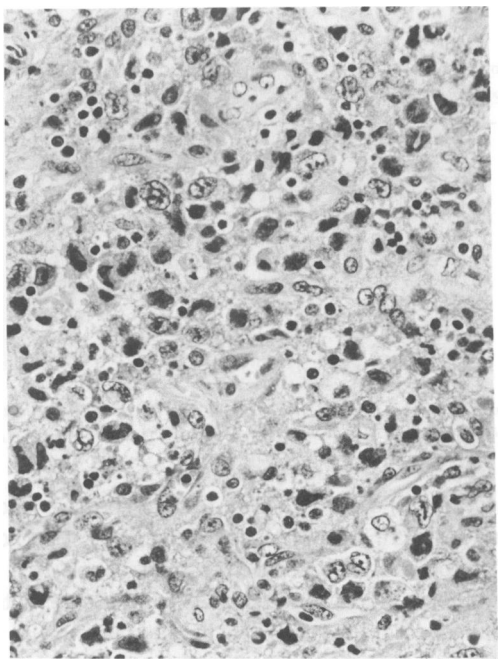

Figure 4. *Case 10. Lymph node. Neoplastic population with a cohesive infiltrative pattern (H&E×250).*

Phenotypic Studies

All cases reacted with at least one CD45 monoclonal antibody (MAb) on tumor cells on frozen sections. Most

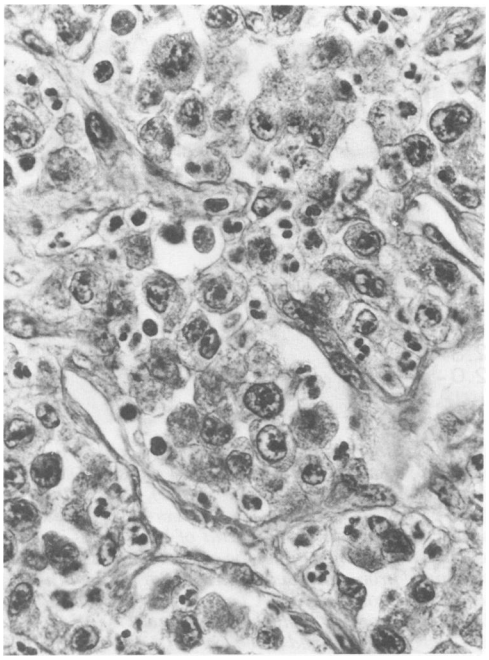

Figure 5. *Case 8. Subcutaneous nodule. Nests of pleomorphic neoplastic cells (H&E×500).*

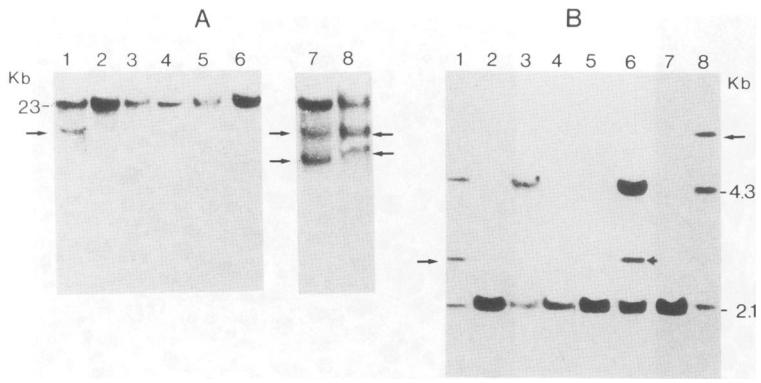

Figure 6. *Southern blot analysis of TCR β and γ chain gene in six cases of MH. Twenty μg of DNA digested with Bam HI* **(A)** *or Hind III* **(B)** *were processed as previously described,[7] and hybridized with the Jurkat 2 clone* **(A)** *and with the M13H60 probe* **(B)** *(4× 10⁶ cpm/ml) for 24 hours at 42°C. The final wash was at 42°C in 0.1× SSC, 0.1× SDS. Lambda DNA restricted with the Hind III enzyme was used as molecular weight marker.* **A:** *Lane 1: case 7. Lane 2: case 3. Lane 3: case 1. Lane 4: case 4. Lane 5: normal lymph node. Lane 6: a B-cell lymphoma. Lane 7: case 6. Lane 8: case 5. The arrows indicate TCRβ rearrangements, while the 23.6 kb band is the germline configuration.* **B:** *Lane 1: case 7. Lane 2: case 3. Lane 3: case 1. Lane 4: case 4. Lane 5: a B cell lymphoma. Lane 6: case 6. Lane 7: normal lymph node. Lane 8: case 5. The arrows indicate TCRγ rearrangements; the 2.1-Kb band is the germline configuration detected by the M13H60 probe, while the 4.3-Kb band is a polymorphic fragment found in some samples.*[11]

cases (8/10) were CD45RO (UCHL1)-positive and all were CD45Ra (F8.11.13)-negative (Table 3). Most, but not all, CD45 epitopes were present on each case (Table 4).

All cases had HLA-DR and CD30 (Ki-1, BerH2) antigen on tumor cells. The intensity of the Ki-1 staining was variable and was confirmed in nine of nine cases on paraffin sections with the Ber-H2 antibody. All cases but one (case 8) were CD25 (IL2-R)-positive. Paraffin sections stained for EMA showed positive staining in 8 of 9 cases, with a great variability both in the number of cells stained and in the staining pattern.

None of the 10 cases reacted on the membrane or intracellularly with a CD3 MAb. We tested all cases with an antibody (βF1) directed against the TCR β chain, which is associated with CD3 on the majority of T cells, and found two cases (cases 8 and 10) positive in the cytoplasm. Two other antibodies recognizing the TCR δ chain, expressed on TCR α/β-negative CD3 lymphocytes, did not react with tumor cells in six cases tested (cases 1 to 5 and 7). CD4 was expressed on seven of 10 cases, the negative ones being both in the TCR-rearranged and in the germline group. CD2 stained a single case out of eight tested (case 2). No other T cell marker (CD 1a, 1b, 1c, CD6, CD7, CD8; 10 cases tested), natural killer (NK) cell marker (CD16, CD56, CD57; six cases tested), or precursor cell marker (CD34; six cases tested) was detected.

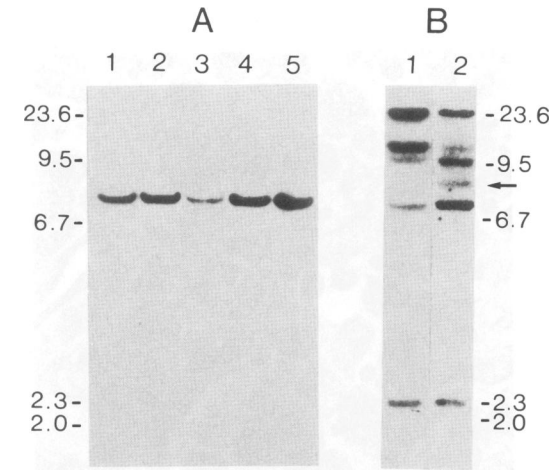

Figure 7. *Southern blot analysis of TCR α chain gene in MH. (See Figure 6 for procedure.)* **A:** *Eco RI digested DNA from case 1 (lane 1), normal lymph node (lane 2), case 5 (lane 3), case 4 (lane 4), a B-cell linfoma (lane 5) were hybridized with K40-B probe. The germline 8-kb band of lane 3 has a decreased intensity, indicating a loss of the alpha segment. Hybridization with control probes did demonstrate that all the lanes carried equivalent amounts of DNA (not shown).* **B:** *Eco RI digested DNA from a normal lymph node (lane 1) and from case 7 (lane 2) were hybridized to the py14 cDNA probe. In lane 2, an additional rearranged band of approximately 8.5-Kb is seen, while the 12-Kb germline band shows a decreased intensity.*

All cases were analyzed for CD5, and four cases were positive; two of them coexpressed TCR β chain (βF1) as well.

The β chain of the leukocyte function associated molecules (LFA family) was expressed alone on three of eight cases. Two cases had both the β chain (CD18), the αL (CD11a), and the αX (CD11c) chains expressed, at least on some neoplastic elements.

Immunostain for myelomonocytic antigens revealed a high number of non-neoplastic macrophages and dendritic cells; this was particularly evident in affected lymph nodes. To discriminate the neoplastic cells from the "background" population, we carefully evaluated the nuclear shape of antigen-positive cells and performed double staining for Ki-67 and CD30 *vs.* T cell or myelomonocytic cell markers (CD68, CD64, βF1, MAC387, lysozyme). CD13 was expressed on six of 10 cases, CD14 and CD68 stained one case each (cases 8 and 9, respectively); none reacted with CD64.

One (case 5) of the nine cases evaluated on paraffin sections was found to react with the macrophage antibody MAC387; the positive case was shown to coexpress at the single cell level CD30 and MAC387 (Figure 8). No such double-stained cells were found in the other eight cases tested.

Table 4. *Pan-leukocyte (CD45) Reactivity: Epitopic Analysis*

CD MAb	45 9.4	45 F10894	45 2B11	45Rb PD7/26	45Ra 10G3	45Ra F81113	45R0 UCHL1
1	+	+	−	+	−	−	+
2	+	+	+w	−	−	−	+
3	+	+	+w	+	−	−	+w
4	+	+	+w	+	−	−	−
5	+	+	−	−	−	−	+w
7	+	+	+w	−	−	−	+
9	(+)	+	(−)	(−)	nd	−	+w
10	(+)	nd	nd	nd	nd	−	+

W, weak; (+) (−), results on paraffin sections.

Lysozyme staining of single atypical cells was present on cases 4 and 6; on double-stained slides, CD30 and lysozyme were found to be on separate cell subsets.

Double staining for the proliferation-associated antigen Ki-67 and other membrane and cytoplasmic markers revealed in cases 1 to 4 occasional small βF1+ proliferating lymphocytes (Figure 9), as well as CD30−, βF1−, CD64−, CD68− residual proliferating germinal center B cells. Ki-67+ tumor cells in cases 1 to 4 were βF1-, CD64-, CD68-negative (Figure 9). CD30 stained all the Ki-67–positive cells and a variable but usually small number of Ki-67–negative atypical cells. Scattered single proliferating histiocytes were found amidst tumor cells and macrophages. The latter were abundant in cases 3, 4, and 5, where they were found to be S-100+, MAC387+/−, CD1−, by comparing frozen and paraffin sections.

None of the 10 cases was found to be positive for a broad panel of B cell-restricted antigens (CD 10, 19, 20, 22, 23), encompassing a wide spectrum of B cell differentiation.

Cases 1 to 8 also were negative for CD28 (Kolt-2), CD36 (OKM5), Mo5, Ki-M4b, CDw65 (Vim-2), CD41a (EDU-3), and an antibody directed against the large and small SV40 T-antigen (Clone 419).

Ultrastructural Studies

Cases 2, 3, 4, 7, 8, and 9 showed similar ultrastructural features (Figure 10); they mainly consisted of large nonphagocytic elements with a polymorphic eccentric nucleus with marginated chromatin, well-developed nucleoli, and deep indentations and lobations. Swollen mitochondria, some lipid droplets, and a variable amount of rough endoplasmic reticulum strands were observed in the cytoplasm. Lysosomes were scarce and of small size; phagolysosomes were not observed. A small juxtanuclear Golgi complex was seldom found. All cases contained a minor component of giant cells with several nuclei or a single hyperlobated nucleus and cytoplasmic features identical to the other tumor cells. Both nonphagocyting and some intermingled actively phagocytizing histiocytes were joined one to another by closely apposed plasma membranes and cytoplasmic projections.

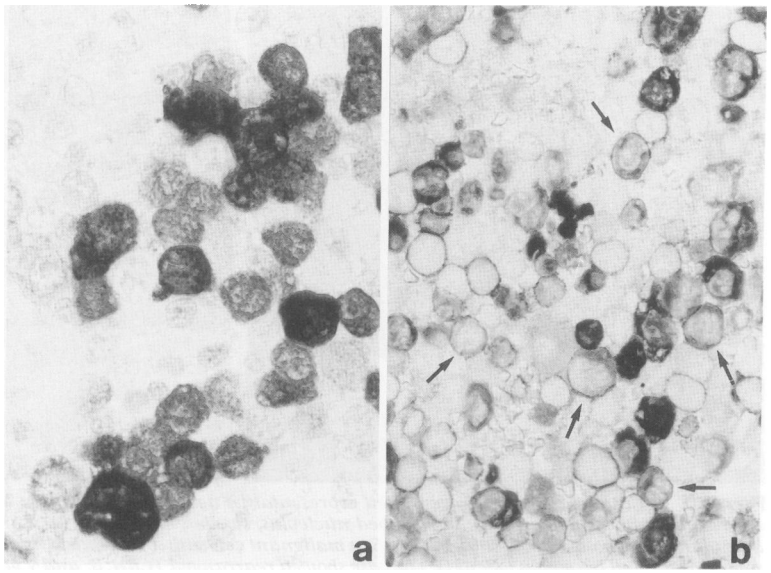

Figure 8. *Case 5.* **a:** *A cluster of MAC387+ large cells (×625).* **b:** *Double staining for CD30 (dark membrane rim, brown in the original picture) and MAC387 (cytoplasmic staining, blue in the original picture) shows double-stained cells (arrows; ×625).*

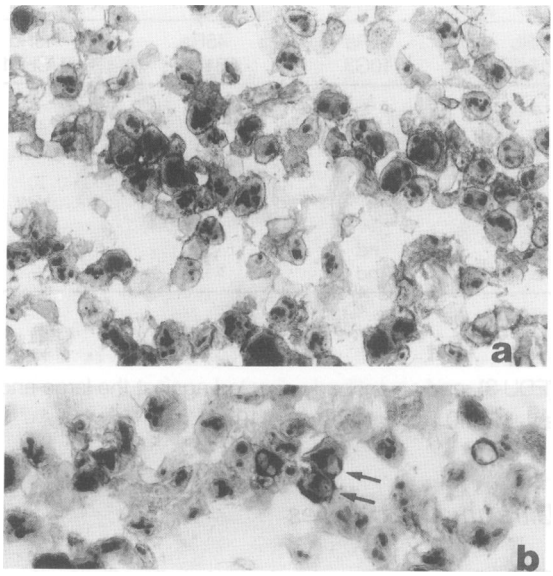

Figure 9. *Case 3.* **a:** *Strongly nuclear Ki-67-positive cells show a rim of membrane Ki-1 antigen (×400).* **b:** *The same tumor cells are not stained by the anti-TCRβ βF1 antibody. A few proliferating T cells (Ki-67+, βF1+) are found (arrows; ×490).*

Discussion

We collected during the last few years 10 cases of nodal and extranodal neoplastic proliferations for which the histopathologic diagnosis of malignant histiocytosis seemed to be appropriate, and deliberately excluded all cases that did not rensemble MH, even though they showed features of NHL of the so-called large cell anaplastic Ki-1+ type, often with carcinomalike appearance.[5] The cases encompassed a variety of clinical features with reference to age, presentation, primary site of involvement, and symptoms.

The spectrum of histopathologic features evaluated on formalin-fixed, paraffin-embedded material were consistent with malignant histiocytosis as generally accepted.[1,3,15]

The phenotype of the 10 MH cases was homogeneous (CD30+, CD45+, CD3−) and, like most of the published cases of Ki-1+ (CD30+) lymphomas,[5,16–23] poorly reactive with a wide range of lineage-specific antibodies.

Poor reactivity with pan leukocyte antibodies (CD45) on paraffin and/or frozen sections was reported,[17,19] particularly when PD7/26 antibody alone was employed. Since this reactivity has diagnostic relevance, we were able to show that there are a few epitopes of the CD45 molecule that are spared and some that are sometimes lost. We found that the 9.4 antibody was the reagent of choice both for paraffin and frozen sections.

Similarly to previously reported cases of anaplastic large cell malignant lymphoma,[16,17,19,21,23] the cases were often EMA+ and CD25+.

We used TCR gene rearrangement as a starting point to sort out the putative T-cell–derived cases and found

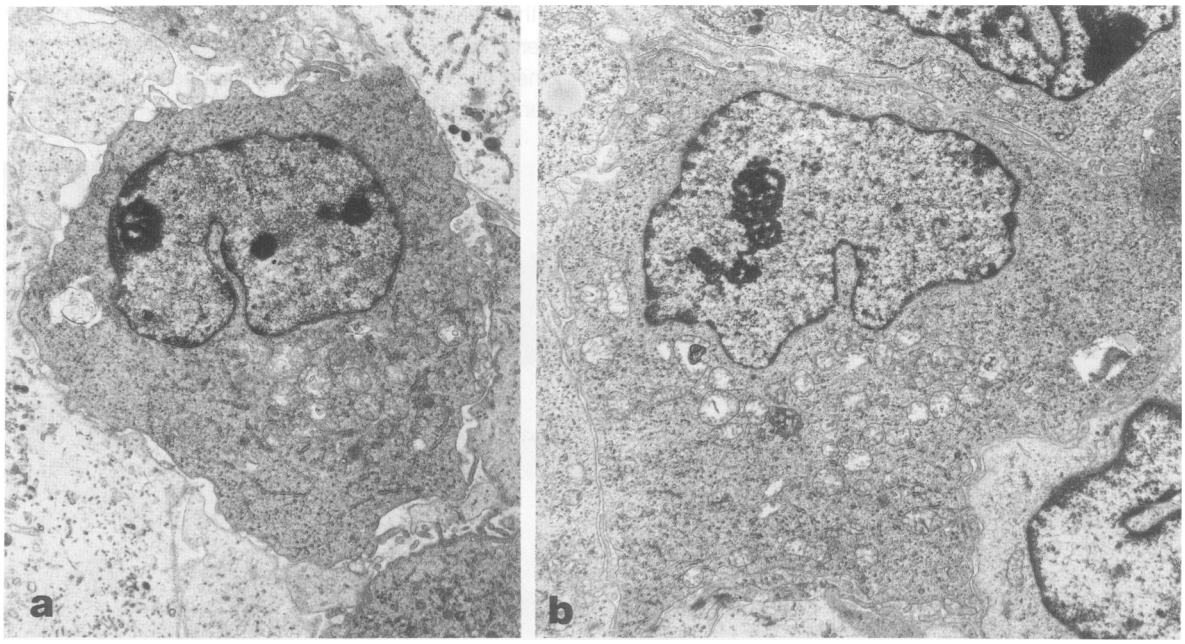

Figure 10. **a:** *Case 3, a poorly differentiated representative neoplastic cell from a MH case with unrearranged TCR and Ig genes with an eccentric nucleus, a well-developed nucleolus, swollen mitochondria, scarce rough endoplasmic reticulum strands, and abundant polyribosomes (×7500).* **b:** *Case 5, a malignant cell with an eccentric nucleus, swollen mitochondria, abundant polyribosomes, and small lysosomes (×7500). The case showed rearranged TCR α, β, and γ genes.*

four cases with rearranged TCR β, α, and/or γ genes. One of these and one additional not evaluated for gene rearrangement had evidence of cytoplasmic TCR β protein. The remaining cases and the germline ones did express neither TCR β nor the alternative TCR δ chain. The CD3 molecule, the heteropolymer associated with α/β or γ/δ TCR, was absent in every case.

The absence of CD3 or of both CD3 and TCR β in TCR β and α gene rearranged T cell lymphomas was reported by Su et al,[24] who showed that CD30+ T cell lines were CD3− and βF1− but all had TCR α transcripts; TCR β transcripts and protein were inducible by phorbol ester stimulation. The absence in our cases of most T cell antigens, including the progenitor cell markers CD34 and CD7, indicates a mature T cell derivation with deletion of T cell antigens due to activation or dedifferentiation,[24] rather than the origin from a precursor-cell leukemia with promiscuous gene rearrangements.[25] The presence of a rearranged TCR α gene in two cases, which is a feature of mature T cells after they have rearranged the β and γ TCR genes, and the absence of Ig gene changes, confirm this hypothesis. We thus may assume that TCR β and γ genes-rearranged cases of MH are indeed malignancies of T-cell lineage.

The absence of JH gene rearrangement and the lack of B cell markers excludes a B cell origin for the 10 cases of MH analyzed. The cellular origin of hemopoietic malignancies that do not display rearrangement either of TCR or Ig genes is still a matter of debate. Two hemopoietic cell lineages, namely the so-called natural killer cells and the bone-marrow–derived macrophages, may give rise to TCR gene germline malignancies analogous to our cases of MH.

The natural killer (NK) cell or "non-MHC-restricted cytotoxic T lymphocyte (CTL)"[26] lacks CD3/TCR proteins and TCR gene rearrangements and bears a unique combination of antigens (CD2, CD8, CD11b and c, CD16, CD56, and CD57).[26] None of the present cases analyzed had an NK-like phenotype, with the only exception of a CD2+, CD16−, CD56−, CD57− case. Furthermore, none of our cases was comparable to the peripheral T-cell lymphomas described by Weiss et al,[27] which were TCR germline, CD2+, CD7−/+, CD5−, CD28+/− extranodal lymphomas, most of them of medium size or of mixed cell type.

Three of our cases with germline TCR and Ig genes displayed markers (CD2, CD4, and CD5) that have been considered T-related.[5,17] We would not rely on these antigens to allocate a TCR gene germline neoplasm into the T-lineage because those antigens have been demonstrated on B cells, myelomonocytic, megakaryocytic, and macrophagic normal cells and their neoplastic counterparts.[28–31]

The histiocytic derivation, which first generated the name of MH, is not supported by the results of the large panel of macrophage-specific antibodies we have employed. Most of the cases with germline TCR gene were unstained; on the contrary, TCR-β–rearranged (T cell?) MH were surprisingly stained by MAC387, CD13, and CD68. The notable exception was the antibody 10.1, directed against the macrophage-restricted FcγR-I (CD64), which has never been tested before on MH and failed to recognize our cases.

We have indeed observed in some cases a few proliferating cells phenotypically identified as histiocytes, but their number was too low to assign them to the malignant clone; they may be regarded as activated nonmalignant cells.

The presence of putative macrophage-restricted markers on CD30+ large cell lymphomas and MH has been reported by some investigators[17–21] and denied by other.[5,16,23] Some of the anti macrophage MAbs-positive lymphomas have been shown to bear rearranged TCR[19] or Ig genes.[20,32] Interestingly, our cases express the same variety of antigens previously reported on CD30+ large cell lymphomas[19,20,32]: some of those MAbs have been described so far as monocyte-/macrophage-restricted (CD13, CD68, MAC387).

Three additional anaplastic large cell lymphomas expressing CD30 and MAC387 have been described,[17] but no detailed phenotypic and molecular biologic data were given.

In summary, MH expresses a limited variety of surface and cytoplasmic antigens, most of them being so-called activation antigens, that can be found on a broad range of cell lineages, even on nonhematopoietic ones (eg, EMA). Most of the function- and lineage-associated antigens are lacking, most likely because of deletion.

Some cases represent the progeny of cells in which the TCR or the Ig gene had been modified at the time of lineage commitment, so that we may be able to assign a given case to a definite lymphoid subset by gene rearrangement analysis. In the case of unrearranged genes, the phenotype, both at the light-microscopy and at the EM level (this paper and Lombardi et al[33]), may not be helpful, because of the lack of function- or lineage-associated markers. Other methods may be needed, such as cell colture, growth factor receptor analysis, and karyotyping.[34]

Note Added in Proof

Negativity for CD45 recently has been reported in routine biopsies from 63 of 165 large cell anaplastic lymphomas (Falini B, et al.) Variable expression of leukocyte-common (CD45) antigen in

CD30 (Ki-1)-positive anaplastic large cell lymphoma. Hum Pathol 1990 (In press).

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